7.

Genetics of *Platypoecilus maculatus*. V. Heterogametic Sex-determining Mechanism in Females of a Domesticated Stock Originally from British Honduras.

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(Plates I & II; Text-figure 1).

The platyfish, *Platypoecilus maculatus*, has two types of genetic mechanisms for sex determination. In members of three natural river populations from Mexico, the males are heterogametic, XY, the females are homogametic, XX; on the other hand, in members of domesticated stocks under observation for 25 years in laboratories in this country and in Europe, the female platyfish are heterogametic, WY (or WZ) and the males are homogametic, YY (or ZZ) according to recent surveys of Gordon (1946b, 1947a, 1950).

In this study the theoretical chromosomal formula of the heterogametic female is being expressed as WY rather than WZ, the homogametic male as YY rather than ZZ, because the author (1946b) demonstrated that the Z chromosome is synonymous with the Y in this species of platyfish. The reason for using WY—YY rather than XY—YY, as suggested sometime ago by Castle (1936), will be discussed later.

MATERIAL.

In the summer of 1947 the Genetics Laboratory received, through the courtesy of Mr. Albert Greenberg, three pairs of the "salt-and-pepper" strain of *P. maculatus* from the Everglades Aquatic Nurseries of Tampa, Florida. Mr. Greenberg said that in 1939 he had collected the original stock of this color variety in streams near the city of Belize in British Honduras. Since that time they have been bred in large, concrete containers for commercial purposes. The platyfish were uniformly marked with prominent black pigment spots on a white background, the males being slightly more heavily spotted than the females. The macromelanophores formed small, discrete pigmented units. Under the lens a few micromelanophores were found between the tight clusters of macromelanophores, but xanthophores or erythrophores, if present, could not be seen.

The posterior area of the caudal peduncle of both males and females had small areas of deeper pigmentation. In some fish of this British Honduras commercial stock the upper and lower parts of the caudal peduncle were darker while in others the central portion was the darker, which made it likely that this strain had some tail patterns. Probably the twin-spot, P^T, was present in some, while others had the one-spot P^O, patterns. The genes for these additional patterns were shown to be autosomal in other strains of platyfish, according to Gordon (1947b).

The salt-and-pepper platyfish will be referred to as the British Honduras commercial or domesticated stock and designated as BH.

GENETIC ANALYSIS.

Each of the three original gravid salt-and-pepper platyfish females was isolated in a four-gallon laboratory aquarium. Their breeding behavior is indicated in Table 1. Females BH-1 and BH-2 produced offspring of essentially similar phenotypes and in the following ratios:

1. Macromelanophore spotting heavy, on white background, like their parents: females, 25%; males, 50%.
2. Macromelanophore spotting faint, on red background, unlike their parents: females, 25%.

Obviously the strain was not true-breeding, for 25% of the brood (all of which were female) were unlike their parents in color. The unlike forms were faintly black-spotted and red, and they resembled the stock mentioned by Kosswig and referred by him and by Breider (1937) to the gene *Sp'R*. This single term *Sp'R* may actually represent two distinct, but closely linked, genes, *Sp'* for macromelanophores and *R* for xanthoerythrophores. For a general discussion of this point see Fraser & Gordon (1929).

The results observed from females BH-1 and BH-2 were obtained again in matings of

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some of their salt-and-pepper offspring; see experiments 4 and 5 of Table 1. When two salt-and-pepper daughters, BH^2A1 and BH^2B1, were mated with their similarly colored brothers, they produced only salt-and-pepper sons; one-half of all the daughters produced were black and white like their brothers, but half of the daughters were faintly black-spotted and red in color.

The results obtained in matings 1, 2, may be explained either by assuming:

1. The female was heterogametic, WY, the male homogametic, YY, as follows:

P_1

Female:		Male:
(W) *Sp'R*/(Y) *Sp*+	×	(Y) *Sp*+/(Y) ++

F_1

Daughters:	Sons:
A. (W) *Sp'R*/(Y) ++	C. (Y) *Sp*+/(Y) *Sp*+
B. (W) *Sp'R*/(Y) *Sp*+	D. (Y) *Sp*+/(Y) ++

2. The male was heterogametic, XY, the female homogametic, XX, as follows:

P_1

Female:	Male:
(X) *Sp'R*/(X) *Sp*+	(X) ++/(Y) *Sp*+

F_1

Daughters:	Sons:
E. (X) *Sp'R*/(X) ++	G. (X) *Sp'R*/(Y) *Sp*+
F. (X) *Sp*+/(X) ++	H. (X) *Sp*+/(Y) *Sp*+

When the two pairs of salt-and-pepper F_1 were inbred, the results in the F_2 were identical with those obtained in F_1 (compare experiments 4 and 5 with 1 and 2). These results failed to indicate conclusively which of the two types of genetic mechanisms for sex determination is in effect in this strain.

Under formula 1: the spotted daughter and its spotted brother chosen for mating may have been individuals marked B and D. These, it will be noted, have the identical genotypes of their parents.

Under formula 2: the spotted daughter and its spotted brother chosen for mating may have been individuals marked F and G. These, it will be noted, are unlike their parents genotypically yet they would produce phenotypically identical offspring in the F_2 as follows:

F_2

Females:	Males:
J. (X) *Sp*+/(X) *Sp'R*	L. (X) *Sp*+/(X) *Sp*+
K. (X) ++/(X) *Sp'R*	M. (X) ++/(X) *Sp*+

The female individual marked K would appear red with faint black spotting. All the rest of the females would be darkly spotted and so would all the males.

Obviously, if enough F_1 matings were set up individually and tested through F_2, one would probably get results which would have eliminated one of the two mechanisms by these methods alone.

The breeding performance of the third salt-and-pepper female platyfish, BH-3, that was born, reared and bred at Tampa, Florida, was much like those of its sisters. But in addition to the types and frequencies of those types which BH-1 and BH-2 produced, BH-3 produced a single, strongly black-banded form, indicated in Table 1, experiment 3, under the phenotype *N*. The gene *N* represents *nigra* a well-known sex-linked color pattern, described early by Bellamy (1922) and studied by Gordon (1937). Beneath the broad band of black pigment and spreading out and radiating away from the black band, the exceptional *N* female had a suffusion of reddish color. This may have indicated that it was carrying the genes *Sp'R* as well as *N*. Apparently the mother of the *N* fish, female BH-3, had mated with more than one male, one of which was +/*Sp*; another was probably *N*/*Sp*.

The results from experiments 1 through 5 were inconclusive in indicating clearly which of the two types of genetic sex-determining mechanisms (XX—XY or WY—YY) was in force in the commercial British Honduras stock (see discussion). It was decided, therefore, to mate one of the British Honduras males with a Rio Jamapa stripe-sided female of a previously genetically tested stock with reference to its sex-determining mechanism. This female was known to have two X chromosomes (XX); and the males of this stock are XY, according to Gordon (1947a).

By chance, the heavily spotted male, BH-14 was chosen for mating with a stripe-sided female (X) *Sr*/(X) *Sr* of the Rio Jamapa population. Phenotypically, the male BH-14 seemed no darker than the other two salt-and-pepper males. The results of experiment 6 in Table 2 indicate that the pair produced 183 adult offspring. Every one of them matured and was a male; approximately half of them were heavily black-spotted (*Sp*) and half were black-banded (*N*). The color patterns of the young were clear cut, as was the ratio in which they appeared. From this mating (Text-fig. 1), it was concluded that the males of the British Honduras stock were homogametic for the sex-determining chromosomes, as indicated below:

Rio Jamapa (Mexico)	×	British Honduras (Commercial)

P_1

Female:	Male:
(X) *Sr*/(X) *Sr*	(Y) *Sp*/(Y) *N*

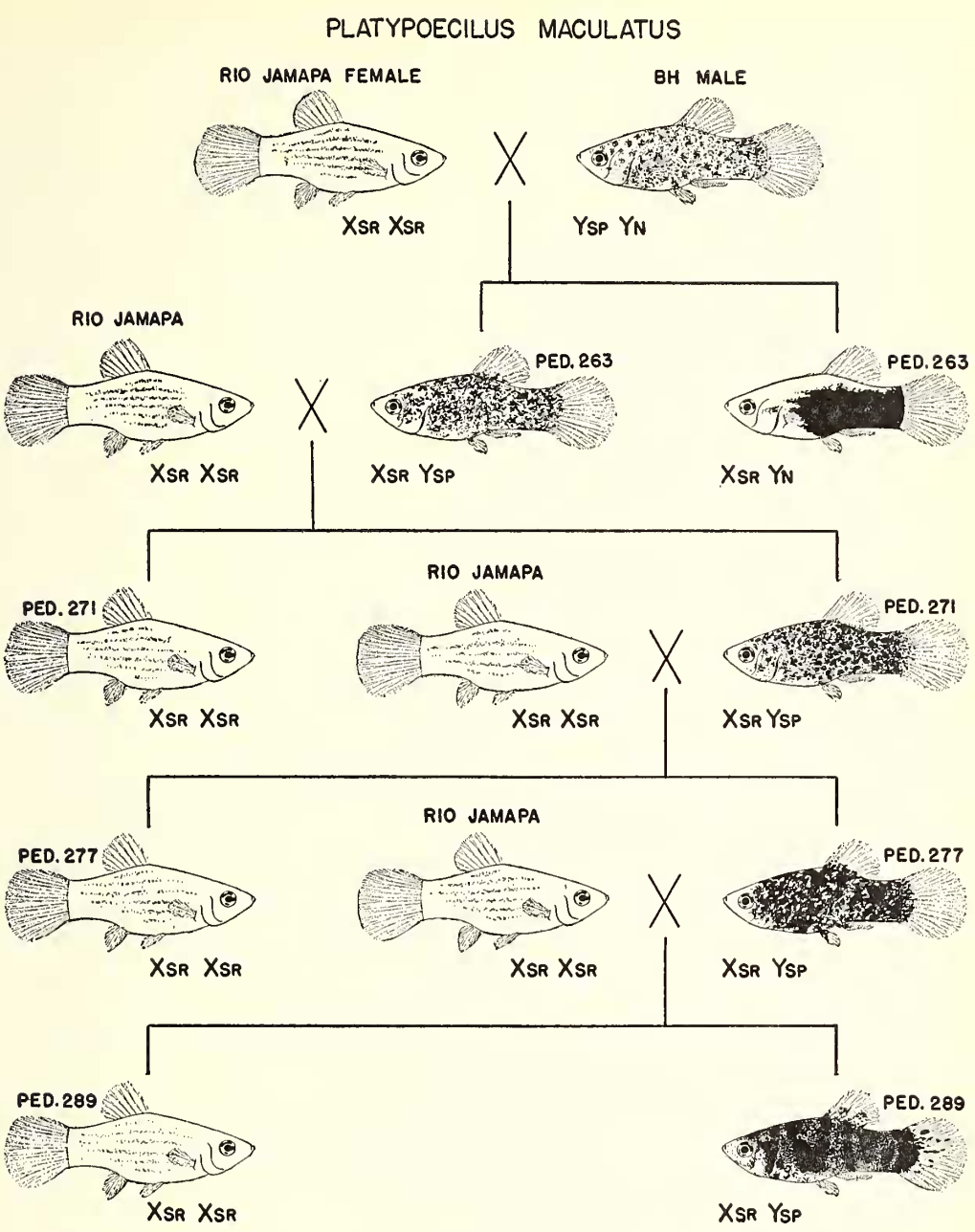
F_1

Pedigree 263

Daughters:	Sons:
None	(X) *Sr*/(Y) *Sp* (X) *Sr*/(Y) *N*

This conclusion was substantiated by a further mating which follows.

A Rio Jamapa-British Honduras spotted



TEXT-FIG. 1. All-male broods produced by mating a male from the domesticated stock of platyfish originally from British Honduras to a female from an inbred stock of wild platyfish originally from the Rio Jamapa in Mexico. The X and the Y represent the sex chromosomes to which sex-linked genes are attached. The first generation is represented by the pedigree number 263 in which two phenotypes appeared: the spotted, *Sp*, and the black-banded or nigra, *N*. The spotted male was back-crossed to a Rio Jamapa female. They produced only stripe-sided daughters and only spotted sons under the pedigree of 271. This type of back-cross mating was repeated, two more times, but the results were the same; that is, only stripe-sided daughters and only spotted sons were produced. This indicates that the father-to-son inheritance may best be explained by the association of the spotted sex-linked gene *Sp* with the Y chromosome.

Note the increasing density of macromelanophore pigmentation in the successive generations of the *Sp*, spotted males.

TABLE 1.

Genetics of the Domesticated Stock of *Platypoecilus maculatus* Originally from British Honduras.

Exp. No.	PARENTS Female	Male	Female	Male	Ped. No.	OFFSPRING *Sp* ♀	*Sp* ♂	*Sp'R* ♀	*Sp'R* ♂	+ ♀	+ ♂	*N* ♀	*N* ♂	*Sr* ♀	*Sr* ♂	Total	χ^2	p
1	BH-1	BH-11	*Sp'R/Sp+*	*++/Sp+*	BH^2A	12	26	10	..	..	..	..	..	..	..	48	0.50	.77
2	BH-2	BH-12	*Sp'R/Sp+*	*++/Sp+*	BH^2B	16	25	15	..	..	..	..	..	..	..	56	0.68	.72
3	BH-3	BH-13 } BH-14 }	*Sp'R/Sp+*	*++/Sp+* } *N+/Sp+* }	BH^2C	33	65	30	..	1	..	..	..	..	..	129	0.17*	.92
4	BH^2A	BH^2A	*Sp'R/Sp+*	*++/Sp+*	BH^3A	18	33	24	..	..	..	..	..	..	..	75	2.62	.27
5	BH^2B	BH^2B	*Sp'R/Sp+*	*++/Sp+*	BH^3B	10	19	8	..	..	..	..	..	..	..	37	0.45	.80

TABLE 2.

Exp. No.	Female	Male	Female	Male	Ped. No.	*Sp* ♀	*Sp* ♂	+ ♀	+ ♂	*N* ♀	*N* ♂	*Sr* ♀	*Sr* ♂	Total	χ^2	p
6	30^{85}	BH-14	*Sr/Sr*	*N/Sp*	263	..	105	..	..	..	78	..	..	183	3.96	.04
7	30^{102}	263-11	*Sr/Sr*	*Sr/Sp*	271	..	63	..	..	..	..	63	..	126		
8	30^{116}	271-11	*Sr/Sr*	*Sr/Sp*	277	..	70	..	..	..	..	60	..	130	0.77	.40
9	30^{119}	277-11	*Sr/Sr*	*Sr/Sp*	289	..	34	..	..	..	..	40	..	74	0.48	.50
10	BH^2A-4	233-13	*Sp/+*	*+/Sd*	276	..	14	11	..	..	..	..	..	25	0.36	.56
11	BH^2A-2	Cp-18	*Sp/+*	*+/+*	274	..	59	23	27	..	..	..	..	109	0.88	.65

BH=Domesticated British Honduras stock of *Platypoecilus maculatus*: 30^8=Eighth inbred generation of the Mexican Rio Jamapa population; *Sp'R*=Genes for evenly scattered macromelanophores and red body coloring; *Sp*=Gene for heavy spotting, macromelanophores in small discrete clusters: *N*=Gene for the nigra pattern, a broad band of macromelanophores: *Sr*=Gene for faint macromelanophores arranged in rows: in the presence of *Sp* or *N* they cannot be distinguished; + = Recessive for sex-linked genes (or no sex-linked patterns visible); *=χ^2 determined without regard to one *N* female.

male hybrid obtained from experiment 6, presumably (X)*Sr*/(Y)*Sp*, was mated back to the homogametic Rio Jamapa stripe-sided female of stock 30 inbred for 10 generations, 30^{10}, (X)*Sr*/(X)*Sr*; these results are reported in experiment 7. All the heavily black-spotted young, of which there were 63, are males while the faintly striped individuals, of which there were 63, are females. The results indicate that the *Sp* gene of the male hybrid was carried on the Y chromosome and appeared in all of the hybrid's sons which presumably have the same genetic constitution as their father, (X)*Sr*/(Y)*Sp*, with reference to the *Sp* gene. This may be indicated as follows:

Rio Jamapa × Rio Jamapa-British Honduras Hybrid

P_1

Female: (X)*Sr*/(X)*Sr* — Male: (X)*Sr*/(Y)*Sp*

F_1

Pedigree 271

Daughters: (X)*Sr*/(X)*Sr* — Sons: (X)*Sr*/(Y)*Sp*

This sort of inheritance may be termed the "father to son," or patroclinous, with respect to the spotted condition *Sp*. A similar series of results was found in the hybrids of wild and domesticated platyfish, according to Gordon (1946b).

In another experiment (8), the back-cross spotted hybrid male of pedigree number 271, (X)*Sr*/(Y)*Sp*, was back-crossed again to a Rio Jamapa female, (X)*Sr*/(X)*Sr*, to determine whether the patroclinous type of inheritance could continue. It did. This time there were 70 black-spotted sons, (X)*Sr*/(Y)*Sp*, and 60 stripe-sided daughters, (X)*Sr*/(X)*Sr*.

Once more a spot-sided male obtained from experiment 8 was back-crossed to a pure stripe-sided female of an inbred stock. And again similar results were obtained; see data in Table 2, experiment 9. All the black-spotted fish were male, of which there were 34, and all the stripe-sided fish were female, of which there were 40.

The patroclinous type of inheritance was in evidence in a series of three back-crosses, and no exceptions were detected.

Before passing on to a discussion of the remaining two experiments in sex determination, it is desirable to declare that there was another purpose in making the series of matings just completed beyond that of studying the sex-determining mechanism. Gordon (1949), in a preliminary announcement, indicated that when a platyfish with a spotted dorsal gene, *Sd*, from the Rio Coatzacoalcos population, was mated with a platyfish from the Rio Jamapa population, of the same species but without the spotted dorsal pattern (+) the Rio Coatzacoalcos-Rio Jamapa platyfish hybrids that inherited the *Sd* gene developed macromelanophores in their dorsal fins within a week after they were born. Not only did the macromelanophores appear

earlier in these hybrids but the pigment cells increased in numbers in the growing animals far beyond the limits known in the normal Rio Coatzacoalcos platyfish.

The second purpose, then, in mating a spotted British Honduras platyfish of the domesticated stock to a member of the wild Rio Jamapa population was to determine if a similar change in the expressivity of the spotted gene, *Sp,* could be induced. The results obtained from experiments 6, 7, 8 and 9 indicate that some important changes in the nature of pigment cell growth did occur in the hybrids of platyfish representing different geographical populations of the same species.

In the first generation hybrids produced (experiment 6) by mating members representing the domesticated British Honduras and the Rio Jamapa Mexican stocks, the spotted fish were considerably darker than either of their parents. When a spotted hybrid, 263-11, male was back-crossed to the Mexican stock (experiment 7), the spotted offspring were darker still. When a darkly spotted back-cross hybrid, 271-11, was back-crossed again to the Rio Jamapa platyfish (experiment 8), there was a further intensification of pigment cell growth in their offspring. When another back-cross was made by mating a double back-cross hybrid, 277-11, to a member of the Rio Jamapa strain (experiment 9), the macromelanophores covered the bodies of the offspring completely, producing intensely black platyfish. Yet no visible pathological conditions resulted.

In order to determine the strength of the W sex chromosome for female determining factors, a mating was arranged using a spotted WY female of the domesticated British Honduras stock, and a spotted-dorsal XY male from a Rio Jamapa population, both the male and female being heterogametic with respect to the sex chromosomes. The mating (experiment 10) may be expressed as follows:

P_1

Domesticated British Honduras	Wild Rio Jamapa (Mexico)
Female (Spot-sided):	Male (Spotted dorsal):
(W)+/(Y)*Sp*	(X)+/(Y)*Sd*

Pedigree 276

F_1

11 Unspotted Daughters	14 Spotted Sons

The mating produced only *two* phenotypes although on the basis of theory there should have been four phenotypic groups to correspond with the four expected genotypes as follows:

Daughters:	Sons:
(W)+/(X)+, Unspotted	(X)+/(Y)*Sp,* Spotted sides
(W)+/(Y)*Sd,* Spotted dorsal	(Y)*Sd*/(Y)*Sp,* Spotted sides and dorsal fin

Apparently the expressivity of the Rio Jamapa platyfish gene *Sd* is reduced to zero and it does not produce a spotted dorsal pattern in the F_1 hybrids between the members of two different populations. In this connection, Gordon (1951) shows that some similar intraspecific platyfish hybrids carry the *Sd* gene but do not show the spotted dorsal pattern. When one of the unmarked (*Sd*) platyfish is mated to a swordtail, *Xiphophorus hellerii,* the platyfish-swordtail hybrids develop a melanosis of the dorsal fin, in response to a reaction between the *Sd* gene and its gene modifiers. When a platyfish-swordtail hybrid with a melanosis of the dorsal fin is back-crossed to a swordtail, some of the young of the back-cross generation develop melanomas of the dorsal fin. An explanation for the suppression of the Rio Jamapa gene *Sd* by genes of the domesticated British Honduras stock is being sought. This subject will be presented and discussed in another paper.

With respect to the reaction of the various combinations of sex chromosomes, it appears that the W of the British Honduras stock has sufficient female-determining genes to override the Y male-determining chromosome of the Rio Jamapa stock. The ratio of females to males is one to one. The consequence of inbreeding members of the hybrid 276 stock may be anticipated by experiments previously described by Gordon (1946a, 1947a). In some F_2 populations the sex ratio is one to one. In others the ratio is three females to one male.

The last experiment (11) in this series concerns the mating of a spotted member of the domesticated British Honduras stock with a recessive male member of the Rio Coatzacoalcos population. The results were quite surprising since experiment 11 was essentially the same as 10. Instead of getting equal numbers of males and females, three males were obtained for every female.

The mating may be expressed as follows:

Domesticated British Honduras	Wild Rio Coatzacoalcos (Mexico)
P_1	
Female (Spotted): (W)+/(Y)*Sp*	Male (Unspotted): (X)+/(Y)+

Pedigree 274

F_1

Daughters:	Sons:
23 (W)+/(X)+	27 (W)+/(Y)+
	59 {(X)+/(Y)*Sp* {(Y)+/(Y)*Sp*

All the spotted (*Sp*) and one-half of the unspotted fish (+) of the F_1 were male while none of the spotted and only one-half of the unspotted ones were female. The W chromosome of the BH (domesticated British Honduras) stock, in association with the Y chromosome of the Rio Coatzacoalcos population, produces males, although females were expected in view of the results in experiment 10. Apparently, then, the Y chromo-

some of the Rio Coatzacoalcos platyfish has a stronger influence in the direction of maleness than the Y of the Rio Jamapa population. This is another example of the hidden genetic differences that exist between the apparently similar platyfish of the same species but from different geographical populations, Gordon (1947b). Experiments between these and other long-isolated platyfish populations are continuing.

Discussion.

The chromosomal formula for heterogametic females used here, WY, is definitive in itself. To use XY for the heterogametic female platyfish would be confusing. Taken out of context, XY may represent a normal male as well as a female. The suggestion of Castle (1936) that the homogametic male be represented by YY has been adopted, particularly because it has been shown (Gordon, 1946b) that the Z chromosome is homologous with the Y. This has been confirmed in some of the present experiments, 6, 7, 8 and 9, which indicate that father-to-son inheritance may be explained by associating the dominant gene for spotting with the Y chromosome.

The three types of sex-determining chromosomes may reflect the presence of three differing sex-determining alleles comparable perhaps to the superior sex gene of *Lebistes*, as suggested by Winge (1932). At any rate, the various chromosomes carrying strong genes for sex are: W for strong femaleness, X for femaleness and Y for maleness. This, of course, is probably an oversimplification of the real conditions. On the whole, Winge's (1934) interpretations, a modified view of Bridges' (1932) theory of genic balance, may be applied to the data obtained with *Platypoecilus*. From his experiments in the field of genetic mechanism for sex determination in *Lebistes*, Winge (1934) and Winge & Ditlevsen (1947) assume that female and male sex-determining genes are distributed over a great many of the autosomes, with superior sex genes in the sex chromosomes. Winge further assumed that, in some individuals, the role of decision with respect to sex may be shifted to the autosomes.

By various systems of selective mating and inbreeding, Winge obtained XY females and XX males, despite the fact that in the guppy the females are normally XX and the males XY. He explains that XY females probably contain many autosomal sex genes which pull strongly in the female direction and in spite of the presence of the Y chromosome, the fish develops as a female. Conversely, the exceptional XX male probably has many autosomal sex genes pulling effectively in the male direction. The first exceptional XX type, once obtained, was recovered after repeated back-crossing. The original XX male was mated to one of its daughters, then to one of its granddaughters and then to one of its great-granddaughters. Obviously, this system of mating involving back-crossing and inbreeding, or some modification of it, could hardly be effective in a natural population in a state of panmixia. For example, Winge & Ditlevsen (1947) report that the normal sex-determining mechanism, XX female, XY male, is reestablished quickly in an aquarium population when the exceptional types, XY females and XX males, are out-crossed:

Female:	P_1	Male:
(X)+/(Y)*Ma*	×	(X)*Li*/(X)*Li*
32 Females:	F_1	35 Males:
(X)+/(X)*Li*		(X)*Li*/(Y)*Ma*

The opposing autosomal sets of sex genes from XX males and XY females apparently balance each other, so that the sex-determining mechanism is again dependent upon the usual X and Y chromosomes, and the male and female offspring are XY and XX respectively.

At best, the exceptional *Lebistes* XX males and XY females are precariously balanced with regard to the sum total of their sex genes. According to Winge, the sex ratio of the offspring produced by mating XX males with XX females is influenced by the season. For example, during the month of April the broods contained almost an equality of the sexes; other times XX males and XX females produced a preponderance of females. Winge & Ditlevson (1947) obtained a sum total of 851 females to only 26 males from many matings over a period of a year. Previously Winge (1934) reported 242 females to 42 males in one series, and 102 females to 42 males in another. From these results he suggested that there is no reason to assume that the pair of deciding autosomes are the same in the two series of XX male *Lebistes*.

In an attempt to identify which specific pair of autosomes in *Lebistes* has become "the new sex-determining chromosomes" in XX males, Winge failed in a number of experiments to associate the new sex mechanism to the autosomal pair carrying the *zebrinus* gene. Winge & Ditlevsen failed, in further experiments, to associate the new mechanism to other known autosomal pairs that carry color genes, of which there are only a few. *Lebistes* has 22 autosomal pairs of chromosomes and one pair of sex chromosomes, or a total of 46 chromosomes.

The conclusions which may be reached from the data on *Lebistes*, as far as they are related to the results from *Platypoecilus*, are: 1. Female and male sex-determining genes are probably distributed over a great many of the autosomes with superior sex genes in the sex chromosomes. The superior sex genes may, in exceptional individuals, be overridden by many autosomal genes with small effects upon sex determination. This may have been the situation in the one XX male platyfish discovered genetically by Gordon (1946a, 1947a) and described histologically by Gordon & Aronowitz (1951). 2. It is unlikely that a pair, any one pair, of autosomes can take over the role of the sex chromosomes in any considerable number of individuals in wild populations in a state of panmixia. 3. It is extremely doubtful that

the switch mechanism by which exceptional XX male and XY female *Lebistes* are produced in the laboratory is the same as the one which produced homogametic male and heterogametic female platyfish in natural populations. Indeed, it may be that female heterogamety arose first in the platyfish of British Honduras and later its isolates, such as populations in Mexico, developed male heterogamety secondarily by a process as yet unknown.

The genetic results obtained from the domesticated strain of platyfish originally taken from British Honduras may best be explained on the basis of assuming that the females are heterogametic and the males homogametic for the deciding genes for sex determination. At first there was considerable doubt with regard to the origin of this strain since in a commercial establishment such as the Everglades Aquatic Nurseries there is a constant shifting of stocks and there is always a possibility of mixing them. A further doubt arose when no comparable color variety of the "salt-and-pepper" platyfish was found in our collection from the Belize River which was taken in 1949 within ten miles of the city of Belize in British Honduras. Nevertheless, after testing the "wild" Belize River platyfish, Gordon (1950) announced that they, too, have a genetic sex-determining mechanism in which the females are heterogametic and the males homogametic. New data, as yet unpublished, revealed that when a Belize River male platyfish was mated to a Rio Jamapa female they produced 65 young, all of which were male. In another similar mating, 240 offspring were obtained of which 239 were male. Since it was previously shown by Gordon (1947a) that the Rio Jamapa platyfish females are homogametic, XX, this is convincing proof that the wild Belize River male platyfish are homogametic with respect to their sex-determining chromosomes, YY.

In an organism which has a stable sex-determining mechanism, and *Platypoecilus maculatus* has such a mechanism despite statements to the contrary, the sex of the individual must be determined at conception. Environmental influences in organisms of this type play a minor role in altering the genetic sex of the individual. This is subject to test. The mating of an XX female with a YY male produced 183 offspring, all of which were male, experiment number 6, Table 2. The sex of these 183 young was determined in a natural way, that is, they were grown to maturity and all of them developed into males at about six months to one year. All of them developed typical gonopodia and other indisputable characters of the male sex. The Rio Jamapa female platyfish that produced them continued to have more young. Forty of these were taken, some when only one day old, others when 28 days old, 73 days old, and so on. None of the young showed any definitive secondary sexual characters externally. They were fixed and their gonads studied. The sex of all of them was determined histologically. All of them had testes, none had any ovarian elements. Thus they were males as far back in their development as histological methods can reveal. The details of this study may be found in the succeeding paper by Chavin & Gordon (1951).

Summary.

1. The domesticated strain known as the "salt-and-pepper" platyfish, *Platypoecilus maculatus*, is allegedly derived from wild platyfish obtained from British Honduras. The fish carry the following sex-linked genes for color patterns: *Sp* for strong macromelanophore spotting; *Sp'* for weak macromelanophore spotting; *R* for erythrophore red coloring of the entire body; *N* for black bands of macromelanophores.

2. The genetic behavior of the sex-linked gene reveals that this stock of platyfish has a type of mechanism for sex determination in which the females are heterogametic, or WY, and the males are homogametic, or YY.

3. When a homogametic male of the domesticated stock from British Honduras, YY, is mated with a homogametic female from the Rio Jamapa in Mexico, XX, all their progeny are male, XY. The genetic mechanism for sex determination in each of these two stocks is stable.

4. The Y chromosome of platyfish from Rio Coatzacoalcos, Mexico, contains stronger sex genes for maleness than the Y of the platyfish of the Rio Jamapa population.

5. There is no evidence that heterogametic male—homogametic female genetic mechanism for sex determination was transformed to the homogametic male—heterogametic female mechanism by the shift of potent sex genes to autosomes, such as has been suggested to account for the development of XX males and XY females in *Lebistes*.

6. The macromelanophores of the domesticated British Honduras platyfish are sensitive to macromelanophore-modifying genes present in the Rio Jamapa platyfish population. The growth of the large black pigment cells is accelerated in their spotted hybrids, but no pathological conditions in them have yet been detected.

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EXPLANATION OF THE PLATES.

Plate I.

Fig. 1. The three types of *Platypoecilus maculatus* of pedigree number BH^2 obtained from the mating of a pair of "salt-and-pepper" domesticated strain of platyfish originally from British Honduras. The heavily-spotted parents looked like the daughter shown uppermost in the figure and the son which is to the extreme right. The two original, heavily-spotted parent color types were recovered. Another type of daughter appeared which was faintly spotted and reddish in color; it is at the extreme left in the figure. The sex ratio obtained from the mating of a pair of "salt-and-pepper" platyfish is as follows: strongly spotted males, *Sp*, 50%; strongly spotted females, *Sp*, 25%; faintly spotted and reddish females, *Sp'R*, 25%. The sex ratio is one to one.

This and the other photographs of living fishes (life size) were made by S. C. Dunton, Staff Photographer, New York Zoological Society.

Fig. 2. Lower: a female platyfish, *Platypoecilus maculatus*, genetically XX, from the Rio Jamapa population in Mexico. Upper: representative male platyfish of the same species developed in a commercial tropical fish hatchery from a "salt-and-pepper" stock originally obtained from British Honduras; genetically the males are YY.

Plate II.

Fig. 3. The two types of males produced by mating a platyfish male of the commercial stocks originally from British Honduras, genetic constitution (Y)*Sp*/(Y)*N*, with a female platyfish from the Rio Jamapa, genetic constitution (X)*Sr*/(X)*Sr*. The entire brood, pedigree number 263, was composed of males; see Table 2. The black or nigra, (X)*Sr*/(Y)*N*, male is shown above and the spotted male, (X)*Sr*/(Y)*Sp*, is shown below.

Note the greater intensity of macromelanophore pigmentation in the progeny.

Fig. 4. The three types of platyfish of pedigree number 274 obtained from mating a spotted female member of the domesticated British Honduras stock to an unspotted male from the wild population of the Rio Coatzacoalcos, Mexico. The sex ratio obtained is as follows: strongly spotted males, *Sp*, 50% (top); unspotted males, + 25% (left); unspotted females, + 25% (right); or three males to one female. Note the greater intensity of macromelanophore pigmentation in the spotted British Honduras-Mexican male hybrid shown here than in the spotted male of the straight domesticated British Honduras stock which is shown in Figure 1; also see Text-figure 1.